Advancing Food Safety: Integration of Environmental Monitoring and Genomics

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Fig 1. in Thompson, A. R. C. (2013) Parasite zoonoses and wildlife: One health, spillover and human activity. *International Journal for Parasitology*, 43:1079-1088.

Impact

- Ecology, Distribution, Persistence

- Combine with genomics (traditional/functional)

  - better source tracking

  - development of preventative controls
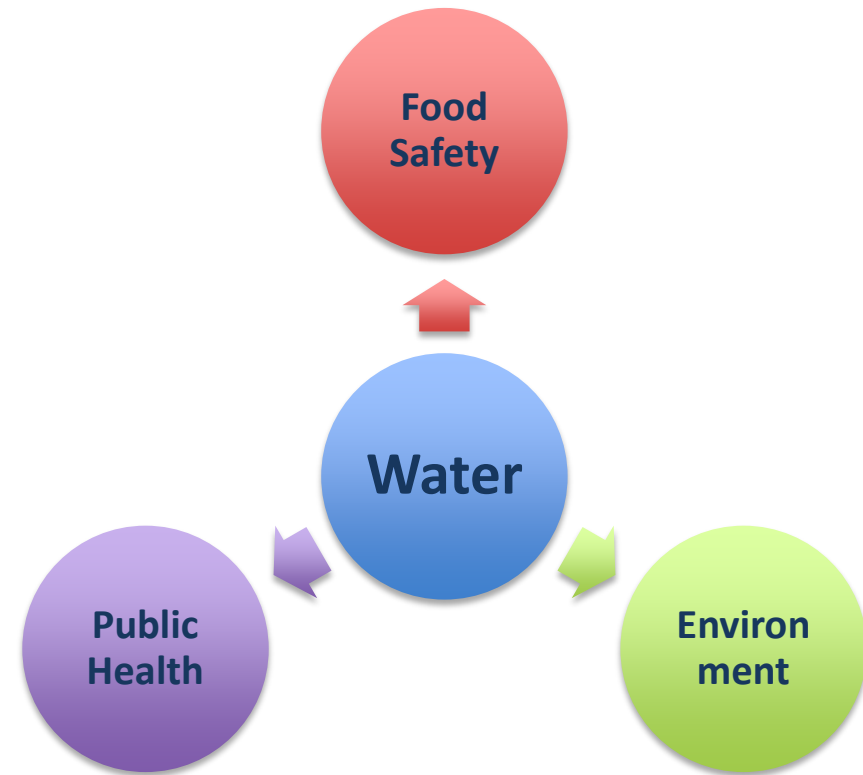
Previous Tomato Outbreaks

FDA

2002	*S.* Newport*	512 cases
	S. Newport	12 cases
	S. Javiana	90 cases
2005	*S.* Newport*	71 cases
	S. Braenderup	76 cases
	S. Enteritidis	77 cases
2006	*S.* Newport*	107 cases
	S. Typhimurium	186 cases
2007	*S.* Newport*	57 cases
2010	*S.* Newport	46 cases
		(retrospective traceback confirmed)
2011	*S.* Newport*	99 cases
2014	*S.* Newport *	283 cases
	S. Javiana	78 cases

First Incident Associated with Maryland Produce
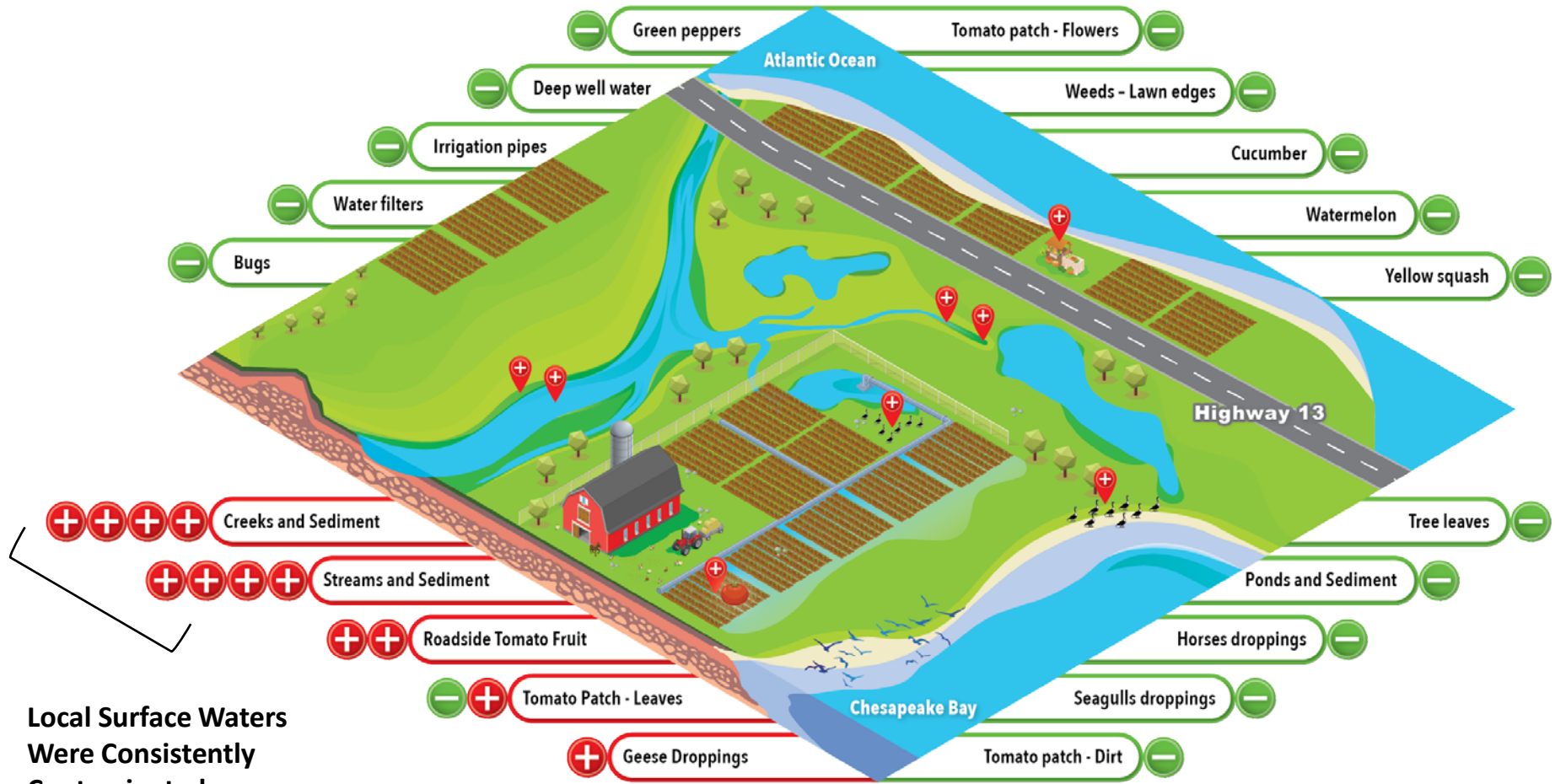
* Same PFGE "pattern 61"

Delmarva Peninsula
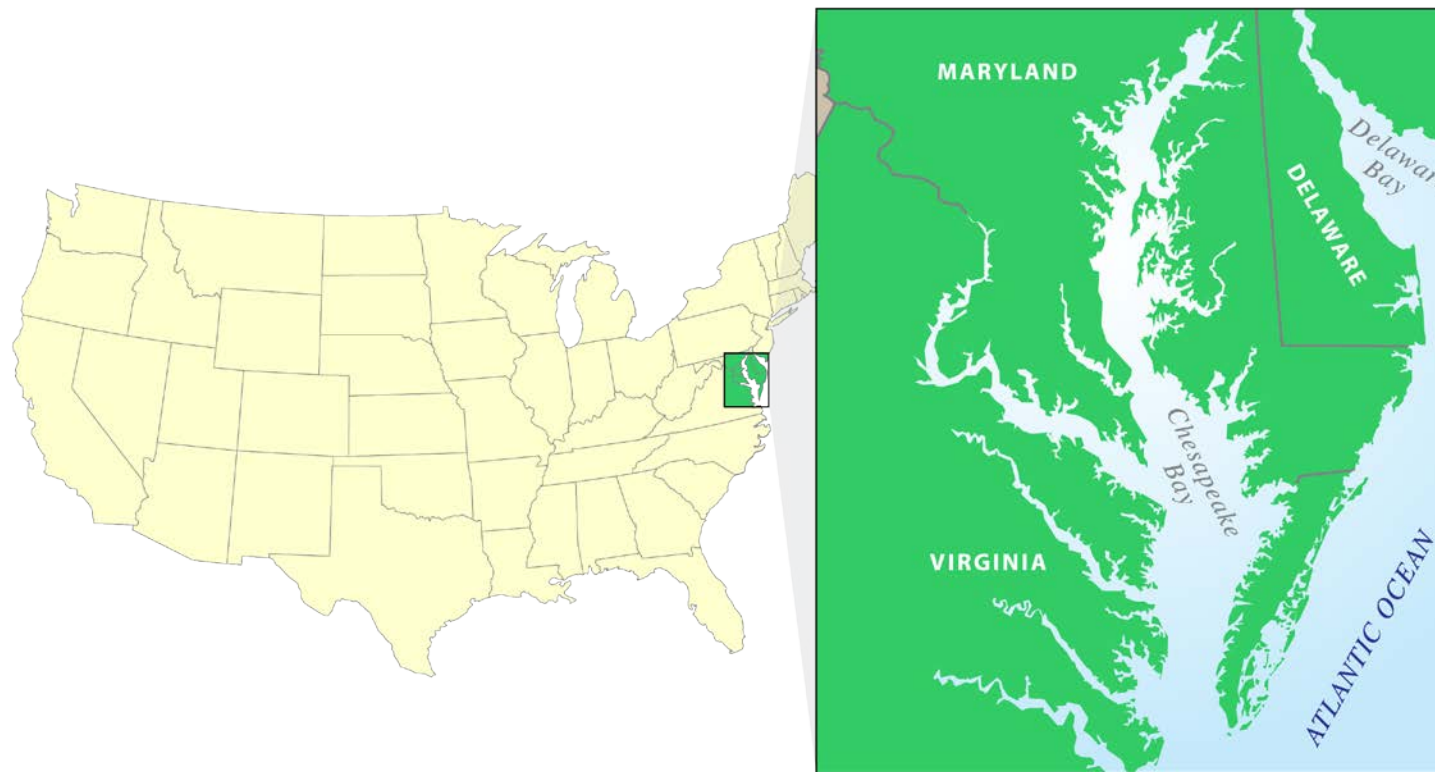
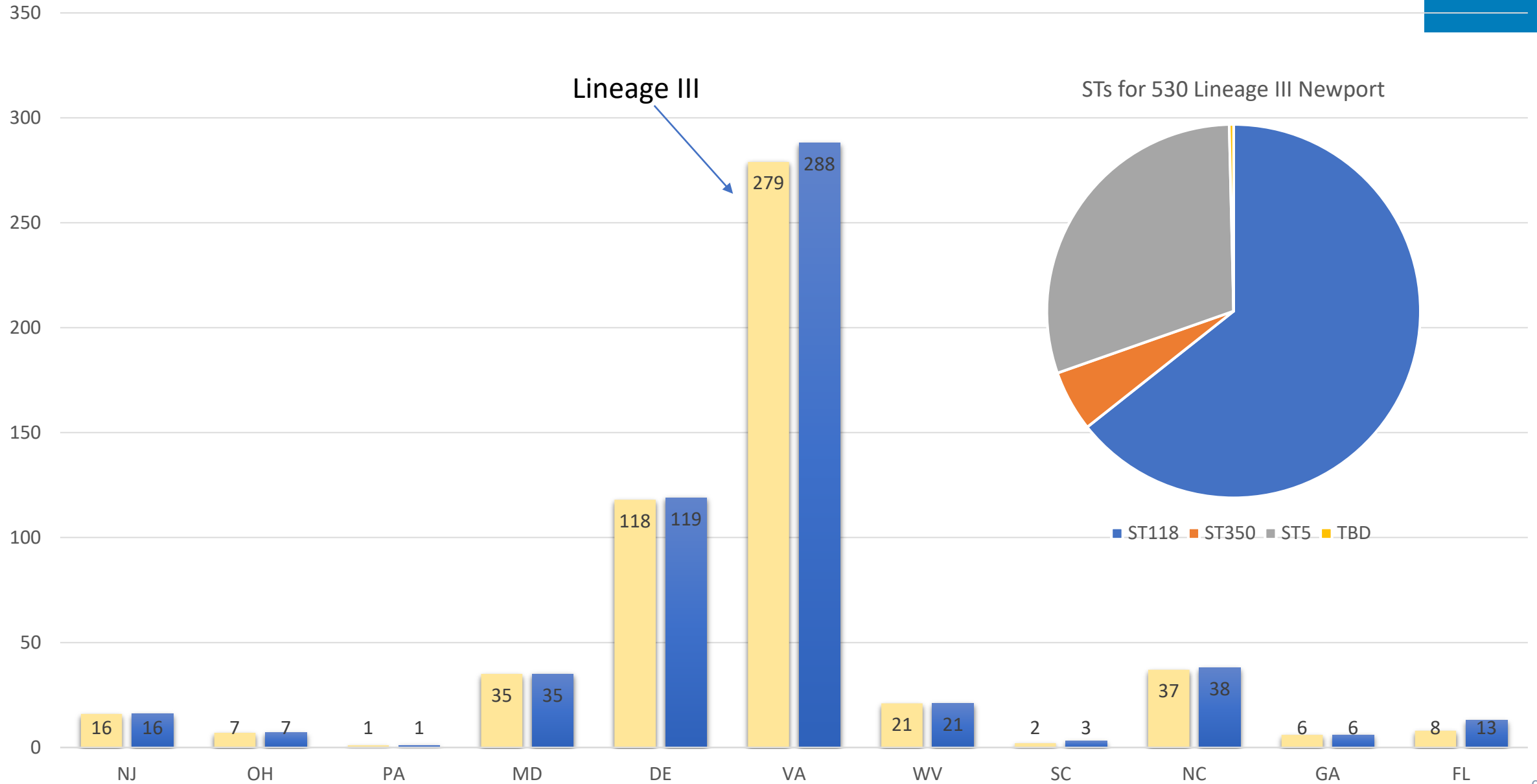# Virginia Tech Eastern Shore Agricultural Research Extension (AREC), Painter, VA

Summary Findings From VA

Local Surface Waters Were Consistently Contaminated Throughout The 3 YR Period

Environmental/Surface water sampling

- Collected samples from 11 different stated on the East Coast

- Some studies were short (*5 sites per state, 4 samplings over 1 summer)

- Some studies were one year or more, longitudinal efforts to the same sites at regular intervals

S. Newport: 547 isolates from 11 states

Lineage III

STs for 530 Lineage III Newport

Bar chart data (yellow, blue):
- NJ: 16, 16
- OH: 7, 7
- PA: 1, 1
- MD: 35, 35
- DE: 118, 119
- VA: 279, 288
- WV: 21, 21
- SC: 2, 3
- NC: 37, 38
- GA: 6, 6
- FL: 8, 13

Pie chart legend: ST118, ST350, ST5, TBD

Pairwise SNP distances

	I	II	III	IV	V	VI
II	834					
III	903	519				
IV	906	519	505			
V	888	580	649	649		
VI	1169	867	932	935	861	
VII	783	475	543	545	472	738
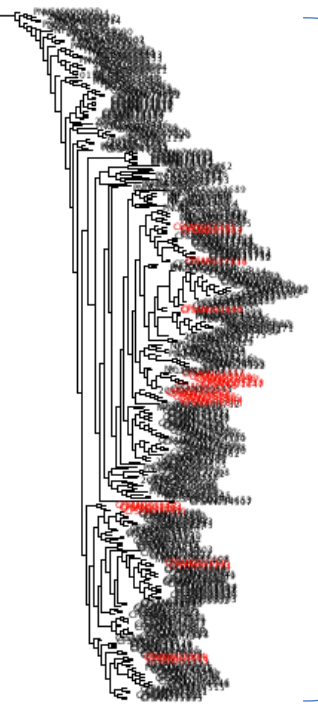
I (344) 0-63 SNPs

Pattern61 DELMARVA Clade
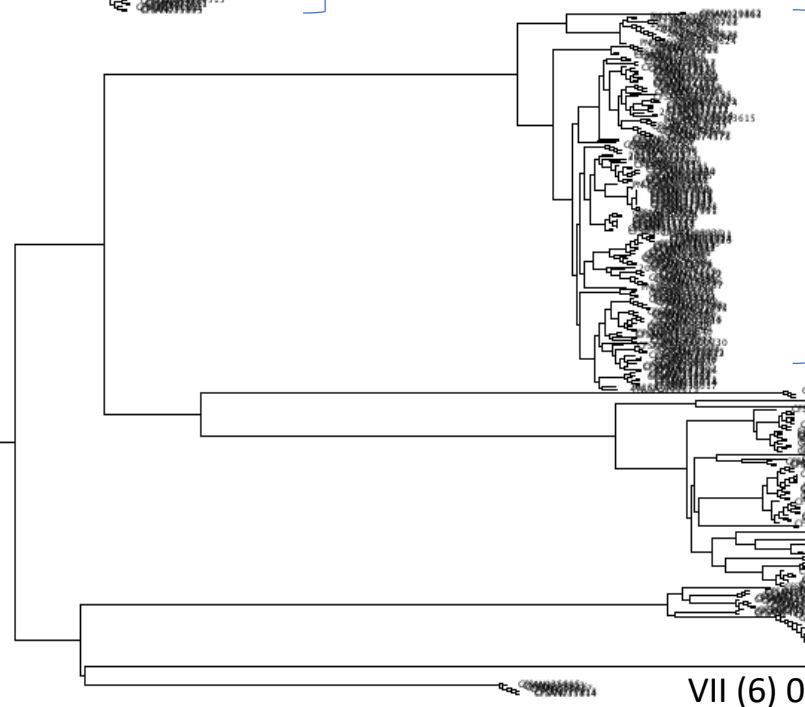
II (189) 0-134 SNPs

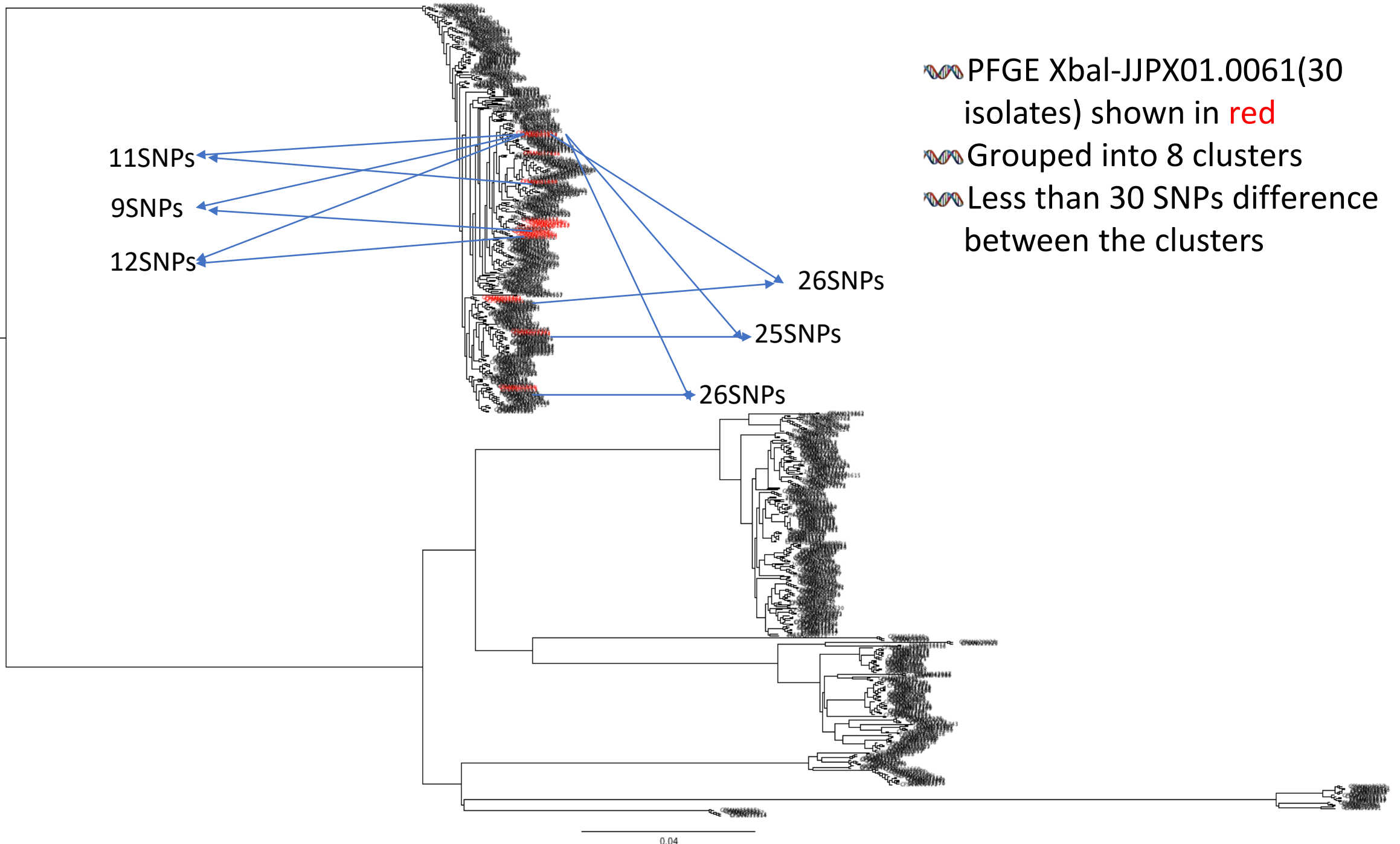**Non Pattern61 DELMARVA Clade***

III (4) 0 SNP

Multi-states clades (152)

IV (94) 0-234 SNPs

V (28) 0-99 SNPs

VI (20) 0-96 SNPs

VII (6) 0-1 SNP

* Two were from SC

11SNPs

9SNPs

12SNPs

26SNPs

25SNPs

26SNPs

PFGE Xbal-JJPX01.0061(30 isolates) shown in red

Grouped into 8 clusters

Less than 30 SNPs difference between the clusters

0.04

FDA's GenomeTrakr

- Distributed network of labs using WGS for foodborne pathogens
- Contributing members:
  - 13   U.S. FDA Labs
  - 11   PulseNet Labs (State Public Health Labs)
  - 5   U.S. Department of Agriculture Labs
  - 7   University Labs
  - 3   Private Labs
  - 2   International Labs (Argentina, Mexico)
  - 1   U.S. Hospital Lab

- All data is submitted to National Center for Biotechnology Information (NCBI)
  - Data curation and bioinformatic support
  - Pathogen Detection
    - Produces daily trees from current SNP clusters

Health > Pathogen Detection

Pathogen Detection BETA

ℹ️ View the recent webinar: 'Introducing the Pathogen Detection Isolates Browser'.

NCBI Pathogen Detection integrates bacterial pathogen genomic sequences originating in food, environmental sources, and patients. It quickly clusters and identifies related sequences to uncover potential food contamination sources, helping public health scientists investigate foodborne disease outbreaks.

Find isolates now!

Examples:
1. Search for isolates encoding a mobile colistin resistance gene and a KPC beta-lactamase
search: AMR_genotypes:mcr* AND AMR_genotypes:blaKPC*
2. Search for Salmonella isolates from the USA
search: geo_loc_name: USA AND taxgroup_name:"Salmonella enterica"

Explore the Data

Species	New Isolates	Total Isolates
Salmonella enterica	18	235,224

Learn More

About

FAQ

Browser Factsheet

Antimicrobial Resistance Factsheet

Antimicrobial Resistance

Contributors

Help

Data Resources
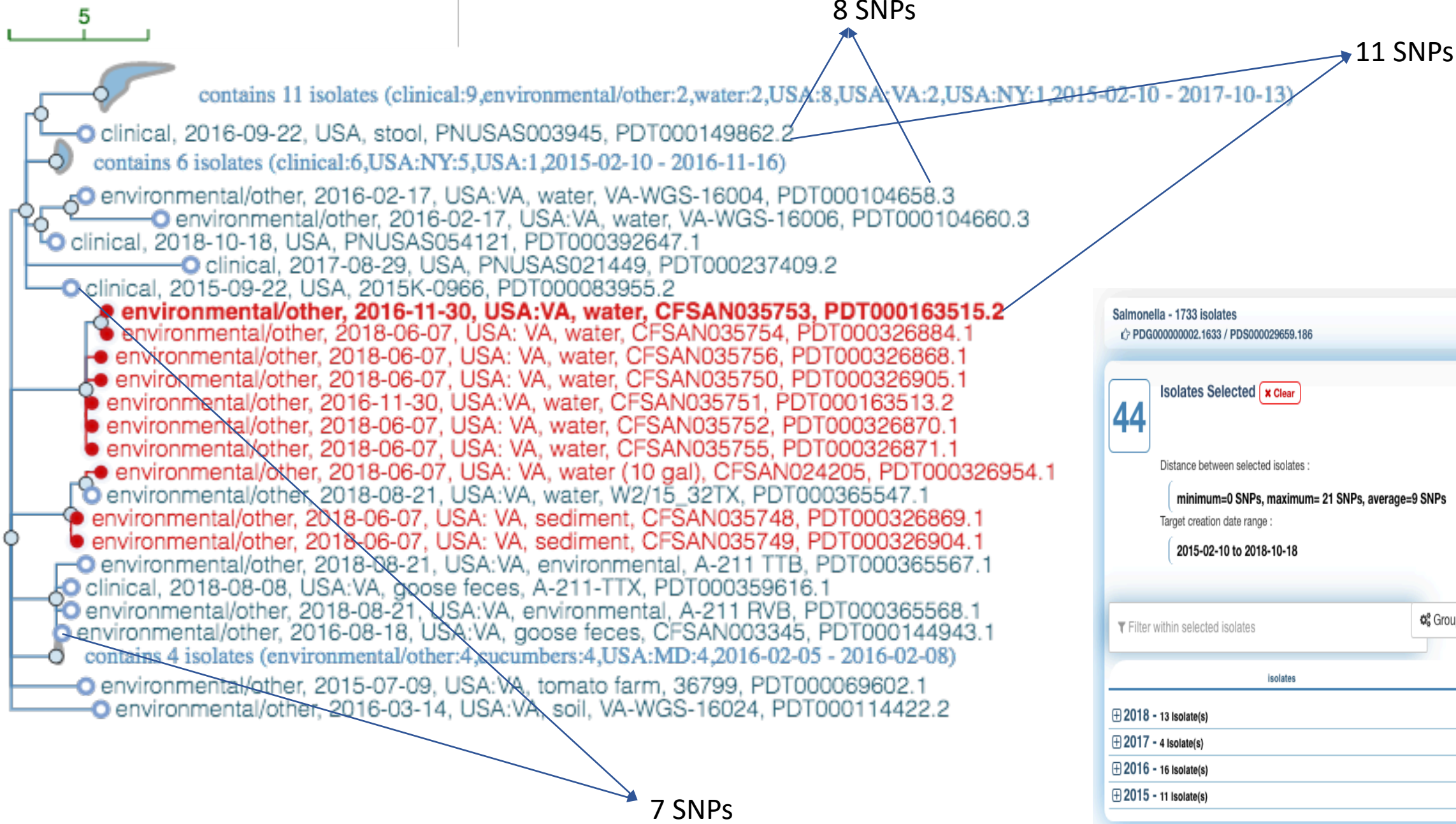
Isolates Browser

Pathogen Detection Reference Gene Catalog

Isolates with antibiotic resistant phenotypes

Download analysis results (FTP)

Submit

13

Antimicrobial Resistance Genes

Acquired antimicrobial resistance gene				
Resistance gene	Predicted phenotype	Identity	Query/Template length	
aac(6')-Iaa	Aminoglycoside resistance	98.63%	438/438	
	Known Mutations			
	Position	Nuc change	AA change	Resistance
parC	parC p.T57S	ACC → AGC	T → S	Nalidixic acid,Ciprofloxacin

ResFinder Analysis

- Threshold of Identity 90%
- Minimum Length 60%

All 530 isolates have the identical Acquired antimicrobial resistance gene profile

All but six isolates share the identical Known Mutation profile

SPI Presence

SPI1	68.50%
SPI2	95.86%
SPI3	95.86%
SPI4	47.27%
SPI5	100%
SPI12	3.57%
SPI13	100%
SPI14	100%
C63PI	100%

SPIFinder Analysis

- Threshold of Identity 90%
- Minimum Length 60%

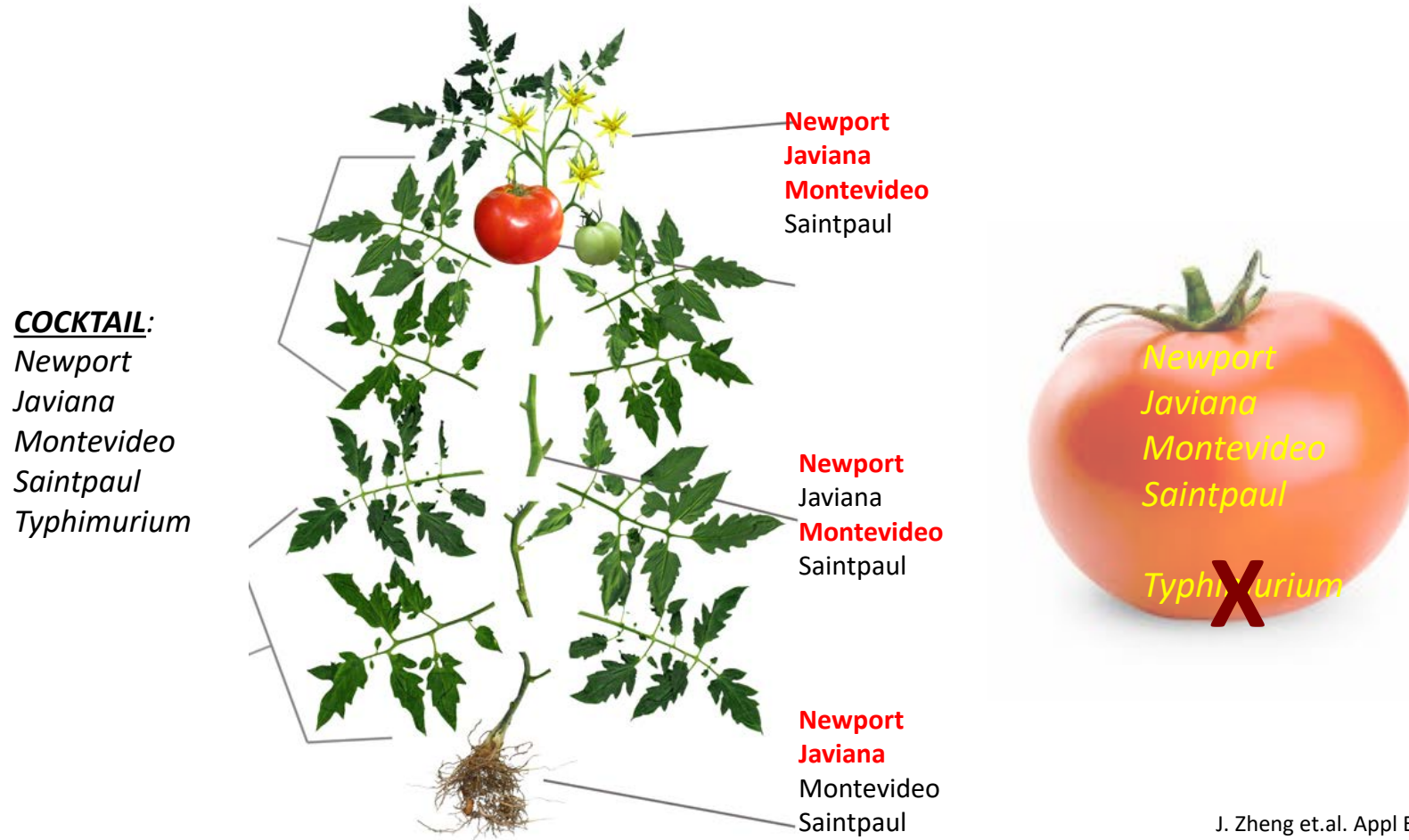Due to the usage of draft genome, false negative are possible

Functions of SPIs identified in Lineage III *S*. Newport

	Size (kB)	GC%	Functions
SPI-1	38.8	45.9	TTSS-1, invasion and proinflammatory responses, Fe2+ and Mn 2+ uptake
SPI-2	39.8	47.4	TTSS-2, intracellular survival; tetrathionate respiration
SPI-3	17.3	47.6	Colonization of GI tract, Mg2+ uptake
SPI-4	23.4	44.8	TISS, colonization of cattle GI tract
SPI-5	7.6	43.6	TTSS-1 and TTSS-2 effector proteins, enteropathogenic responses
SPI-12	6.3	49.92	TTSS-2 Effector
SPI-13	19.5	48.1	Virulence in chicks
SPI-14	8.7	41.4	Virulence in chicks
C63PI (Centisome 63)		SPI locates at centisom 63	

Clade defining SNPs

| Position	Locus tag	Product	Ref. base	Ref. codon	Ref. res.	SNP type	New base	New codon	New res.
		Clade VI							
228329	SEENLE01_001045	hypothetical protein	G	CTG	L	synonymous	A	CTA	L
671544	SEENLE01_003170	flotillin	T	GTA	V	nonsynonym	A	GAA	E
1068222	SEENLE01_005140	multidrug export protein EmrA	G	GCC	A	nonsynonym	A	ACC	T
1252656	SEENLE01_006025	pyridoxine 5'-phosphate synthase	C	ACC	T	synonymous	T	ACT	T
2335611	SEENLE01_011225	methyl viologen resistance protein SmvA	C	GCC	A	synonymous	T	GCT	A
2337186	SEENLE01_011235	porin	G	CTG	L	synonymous	A	CTA	L
2577044	SEENLE01_012435	threonine--tRNA ligase	C	CGC	R	nonsynonym	T	TGC	C
3217918	SEENLE01_015790	glycosyl transferase	C	CCT	P	nonsynonym	T	TCT	S
3517731	SEENLE01_017335	multidrug ABC transporter permease/ATP-binding protein	C	GCG	A	nonsynonym	T	GTG	V
3517802	SEENLE01_017335	multidrug ABC transporter permease/ATP-binding protein	G	GAG	E	synonymous	A	GAA	E
4106327	SEENLE01_020075	PTS sugar transporter	C	TCA	S	nonsynonym	T	TTA	L
4106947	SEENLE01_020080	Fis family transcriptional regulator	G	TGG	W	nonsense	A	TAG	*
4457460	SEENLE01_021735	chemotaxis protein	G	GAT	D	nonsynonym	T	TAT	Y
4457520	SEENLE01_021735	chemotaxis protein	G	GTG	V	nonsynonym	A	ATG	M
		Clade IV							
1321245	SEENLE01_006320	outer membrane protein assembly factor BamB	A	ACC	T	nonsynonym	g	gCC	A

Pathogen Colonization and Internalization Studies

Newport
Javiana
Montevideo
Saintpaul

***COCKTAIL*:**
Newport
Javiana
Montevideo
Saintpaul
Typhimurium

Newport
Javiana
Montevideo
Saintpaul

Newport
Javiana
Montevideo
Saintpaul

Newport
Javiana
Montevideo
Saintpaul

*Typhimurium* X

J. Zheng et.al. Appl Environ Microbiol. 2013 Apr;79(8):2494-502

- 70% of inoculated blossoms resulted in *Salmonella* isolated from within or on the developing fruit
- Newport, Javiana, Montevideo, and Saintpaul were all recovered from within developing tomatoes
- *Salmonella* Newport internalization through root system was internalized to stem (20%) and fruit (5%)

M. Erhardt, K. Namba, and K. T. Hughes Cold Spring Harb. Perspect. Biol. 2010; 2:a00099
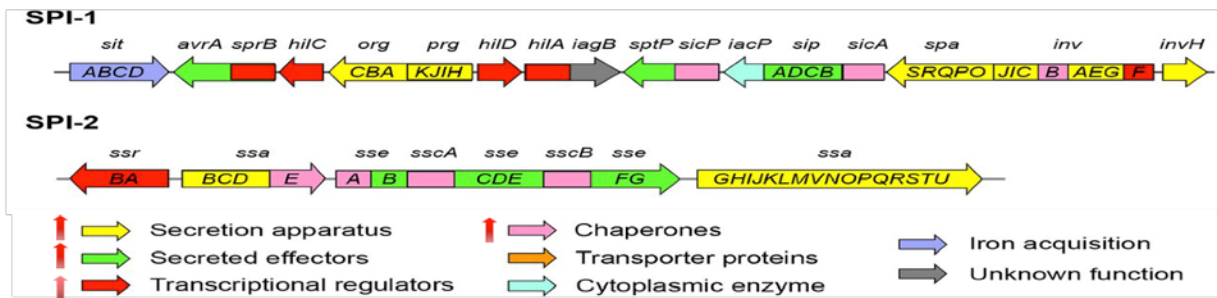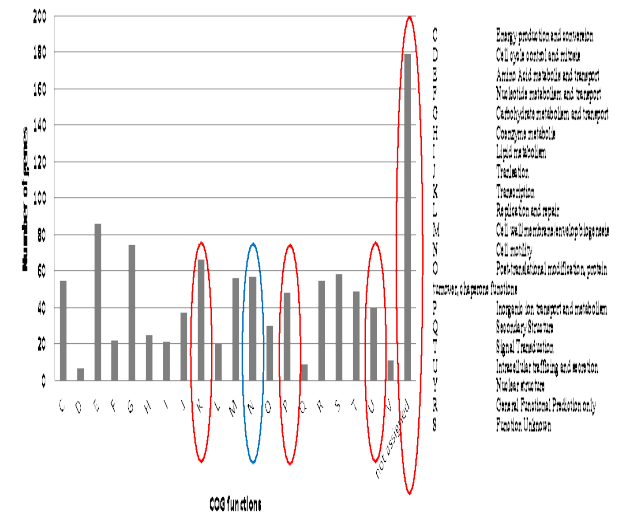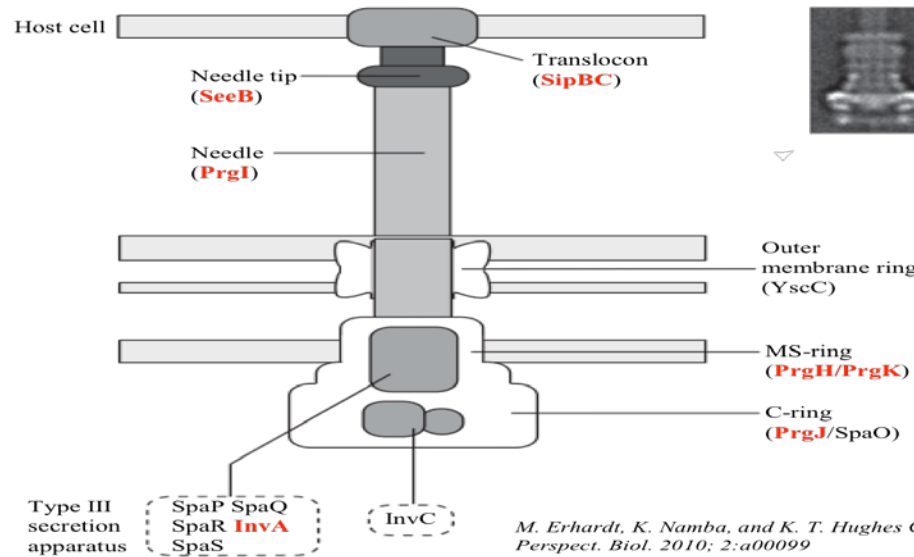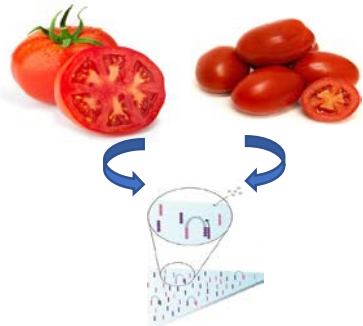
Anna Fàbrega, and Jordi Vila Clin. Microbiol. Rev. 2013;26:308-341

Salmonella Adaptations of particular interest to food safety specialists

(1) Thermal tolerance

(2) Dessication resistance

(3) Osmotic/Ionic tolerance

(4) Quat resistance

(5) Chlorine resistance

(6) Biofilm persistence

(7) Surface adherence

(8) Antibiotic resistance

(9) Antimicrobial resistance

(10) Ecological fitness

(11) Heavy metal resistance

(12) Metabolic persistence

(13) Enhanced hydrophobic fitness

(14) Produce invasiveness

(15) Flower invasiveness

(16) Root system invasiveness

(17) Acid resistance

(18) Surface water fitness

(19) In vivo plant migratory fitness

(20) Soil fitness

(21) Capsaicin resistance

(22) Swarming

(23) Trans-ovarian poultry colonization

(24) Fecal persistence (poultry)

(25) Yolk content invasion

(26) Multidrug resistance

(27) External amoeba harborage

(28) Internal amoeba harborage

(29) Acyl-homoserine lactone (AHL)

(30) KatE stationary-phase catalase

(31) In vivo migratory fitness

(32) RDAR phenotype

(33) The 'Weltevreden' type

(34) Persistence within the tomato**

Key Conclusions

- *Salmonella* Pattern 61 and other clinically relevant isolates found consistently in waters and sediments
- Environmental waters and sediments are potential reservoirs for *Salmonella*
- Agricultural practices related to the use of contaminated surface waters are highly likely to introduce *Salmonella* onto crop plants
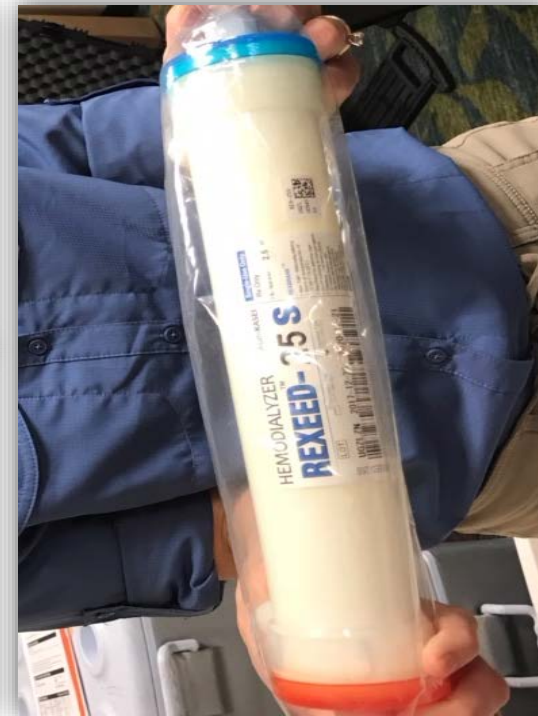
Water Sampling Methodologies

Traditional Grab
Sample
1 L

Modified Moore
Swab (mMMS)
10 L

Hollow Fiber
Ultrafiltration
100L

Pros/Cons:
-Ease of collection/processing
-Cost

-Sensitivity of detection
-Equivalent sample volume

Acknowledgements

Dr. Guojie Cao

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    Dr. Jie Zheng

    Christina Ferreira

    Elizabeth Reed

CFSAN Core Sequencing group

CFSAN Bioinformatics group

Questions???

If tomatoes are classified as a fruit...

Isn't ketchup technically a smoothie?